PLANT PHYSIOLOGY.—*Responses of certain fungi, particularly* Trichoderma *sp., to light.*[1]

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The importance of light for growth and development of fungi is evident from the widely scattered observations in the literature and the increasing number of recent studies. Its importance for processes other than photosynthesis is well demonstrated for higher plants (Hendricks and Borthwick, 1955) and has also been demonstrated for ferns (Mohr, 1956) and algae (Finkle, Appleman, and Fleischer, 1950; Killam and Myers, 1956). Owing to fundamental differences in structure and development among these groups of plants, their responses to light are expressed quite differently. Despite the differences, careful studies of the way light acts to induce each response can show whether the same or different photoreactions are involved.

The purpose of the present study was to explore the types of response of several fungi to light and to study in detail the response of one of them which appeared to be best suited for this type of investigation—viz., *Trichoderma* sp.

MATERIALS AND METHODS

Potato-dextrose-agar was generally used as the culture medium, and the cultures were grown at 21° C. Test tubes were used as culture vessels, except for *Trichoderma* sp., which was grown in petri dishes. The cultures were standardized by starting each from a single spore when possible—otherwise from a hyphal tip. For qualitative observations, photographic records and visual estimations were made. For the quantitative determinations, spores were counted or percent transmissions of light by the spore suspensions were determined with a spectrophotometer.

Cool, white, fluorescent tubes, at distances of 48 or 100 cm, gave illuminances of 78 or 34 f.c., respectively. For isolation of blue and red wavelength regions, two layers of blue or two layers of red cellophane were used with these tubes or with blue or red fluorescent tubes at the same distances when higher energies were desired. The fluorescent tubes were used as blue and red sources because they emitted very little of the far-red wavelengths (7,000 to 8,000 Å). The far-red wavelength region from four incandescent-filament lamps (125-watt) was isolated by a filter consisting of two layers each of blue and red cellophanes (Piringer and Heinze, 1954). Radiant energy from such lamps was rich in the far-red wavelengths. Radiant energy was varied either by varying the distance of the cultures from the source of light or by interposing neutral filters consisting of different numbers of layers of cheesecloth. The action spectrum for *Trichoderma* sp. was determined with the spectrograph described by Parker, Hendricks, Borthwick, and Scully (1946).

RESULTS

Twelve species of fungi were grown in light and in darkness and examined for responses, such as production of sexual and asexual fruiting bodies, growth of mycelium, and formation of pigments. Cultures of one of these species, *Mucor* sp., formed a mat of mycelium in the dark and under all conditions with light, but it did not fruit under any condition. Lack of fruiting suggested that there was lacking in the culture medium some compound necessary for the further development of the fungus. The other 11 species, including *Trichoderma* sp., which is discussed in detail in the last part of this section, showed one or more responses to light.

Stemphylium sp. (a yellow mycelial strain) did not sporulate in 4 weeks in darkness. This finding verified earlier work on other species or isolates of *Stemphylium*

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(Weber, 1930; Hannon and Weber, 1955) which also required light for sporulation. When cultures were grown on potato-sucrose-agar at 21° C., maximal spore production was obtained when light was given 26 to 72 hours after inoculation with a single spore. For sporulation, darkness prior to exposure to light was apparently unnecessary and, once initiated, spores apparently matured in light or darkness. After 50 hours of growth of the cultures in darkness, the shortest period of continuous white fluores-

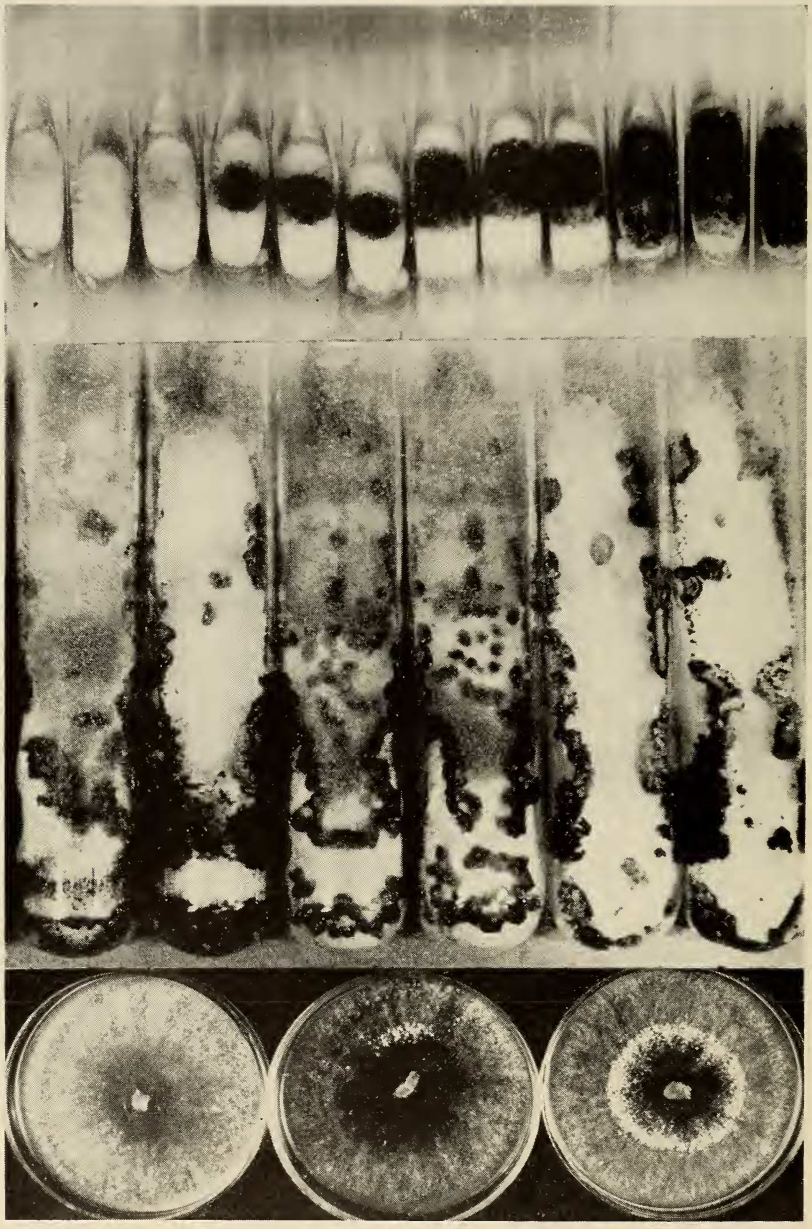

FIG. 1.—Top row: Sporulation of *Stemphylium* in response to various durations of white fluorescent light of 78 f.c. intensity. From left to right groups of three tubes received no light, 48 hours, 72 hours, and continuous light. Middle row: Formation of sclerotia in cultures of *Botrytis gladiolorum* in response to blue (first tube), far-red (second tube), red (third and fourth tubes), and darkness (fifth and sixth tubes). Bottom row: Sporulation of *Trichoderma* sp. in response to darkness (left), 1-minute exposure of sector at top of culture (center), and 1-minute exposure of whole culture (right) to 34 f.c. of white fluorescent light.

cent light (34 f.c.) that induced sporulation was 18 to 23 hours and the number of spores increased with duration (Fig. 1, upper row) and intensity of light. The least amount of white fluorescent light (78 f.c.) that induced spore formation, when given in daily cycles, was 1 hour per day for 4 or 5 days. With continuous white fluorescent light, saturation for sporulation was obtained in 7 days. During that period the intensity of the light was alternated (500 f.c. for seven hours and 78 f.c. for 17 hours). Red and far-red wavelengths were ineffective or much less effective for inducing sporulation than were the shorter wavelengths of the visible spectrum.

Mycelial growth of *Stemphylium* was stimulated by about 5 f.c. of continuous white fluorescent light at 21° C., but was retarded at 28° C. All wavelengths of the visible spectrum appeared to be effective for mycelial growth. In darkness the culture medium and the mycelium were yellow. In light there was a negative correlation between the number of spores and the intensity of yellow color; in no case was an irradiated culture as deep a yellow as those grown in the dark. The concentration of the yellow pigment in the medium was high under conditions unfavorable for spore formation—exposure to red, far-red, or low-intensity white light, increase in sugar concentration of the medium, or a temperature of 28° C. The pigmentation of the mycelium and medium might have been directly dependent on light or indirectly dependent through the action of light on growth and sporulation of the organism.

Cultures of *Botrytis gladiolorum* Timmerm. showed three responses to light—sporulation, ridging, and formation of sclerotia. The number of spores formed in response to exposure to white fluorescent light (78 f.c.) increased with duration of exposure from 7 to 70 hours. Mycelial ridges, consisting of aggregations of erect, aerial, spore-bearing hyphae, alternating with areas of sparse, prostrate hyphae bearing few spores, occurred in cultures exposed to certain daily alternations of 34 or 78 f.c. of white fluorescent light and darkness which followed not more than 3 days of darkness. Ridges were formed in cultures that received at least eight but not more than 23 hours of light in 24-hour cycles for 4 or 5 days. Ridges were not formed in cultures in continuous light or darkness. Continuous light resulted in a dense uninterrupted layer of spores and darkness resulted in a thick layer of nonsporulating mycelium. Blue light promoted spore formation and ridging.

Sclerotia did not form in cultures in continuous white fluorescent light (78 f.c.), but a few formed in continuous darkness. The best conditions for the formation of sclerotia were provided by a 30-minute irradiation with white fluorescent light after the cultures had grown in darkness 4 days. The cultures were returned to darkness and observed 8 days later. In three experiments, that tested the relative effectiveness of red and blue lights, more sclerotia were formed in cultures exposed to red light; the response to blue light was about the same as to darkness (Fig. 1, middle row). A promotive effect of red light has not been previously reported, but blue light has been observed to be inhibitory for sclerotium formation by *Botrytis cinera* (Reidemeister, 1909), five species of *Aspergillus* (Tatarenko, 1954), and *Verticillium alboatrum* (McClellan, Borthwick, Bjornsson, and Marshall, 1955).

Sporulation of *Curvularia tribolii* (Kauff.) Boed. and a dark mycelial strain of *Stemphylium* sp. was promoted in cultures exposed to continuous white fluorescent light (78 f.c.) as compared with that of cultures in darkness.

Penicillium gladioli McCull. et Thom. produced an abundance of aerial mycelium but no spores in continuous far red or in darkness. Cultures grown in continuous red light formed a few spores but considerable aerial mycelium; those grown in blue light formed an abundance of spores but no aerial mycelium. These results agree with earlier reports on several species of *Penicillium* (Tatarenko, 1954).

Stromatinia gladioli (Drayton) Whez. formed a greater number of sclerotia when grown in continuous white fluorescent, blue, or red light for 4 weeks than in continuous darkness. Blue light appeared to be the most promotive.

Rhizoctonia carotae Rader produced sclerotiumlike bodies when grown in continuous

white fluorescent light (34 f.c.) either unfiltered or filtered with two layers of blue or red cellophane, but did not form these structures in the dark.

Diplodia sp. from cotton did not form pycnidia during 27 days in the dark, but produced these structures under all light treatments. Cultures in red light formed fewer, larger, and longer-necked pycnidia than did those in either blue or white fluorescent light (78 f.c.).

Rhizopus sp. formed sporangia abundantly when exposed continuously to unfiltered white fluorescent light or filtered blue light from blue fluorescent tubes, but both of these kinds of light depressed sporangiophore elongation. Cultures in red light or darkness formed long sporangiophores, only a few of which developed sporangia. Cultures grown in an alternation of eight hours of white or blue fluorescent light and 16 hours of darkness produced fewer sporangia but somewhat longer sporangiophores than did cultures in continuous light.

Lenzites trabea (Fr.) Fr. did not form basidiocarps during 12 months in darkness. Cultures, each started from a hyphal tip and grown in continuous white light for 4 weeks, also failed to produce fruiting bodies. However, if such cultures were given an additional 6 weeks in the light, small, incompletely developed basidiocarps were formed during an additional 4 weeks in darkness. They were also formed when filtered red or blue fluorescent light—but not far-red—was used. Cultures grown on malt agar gave the same results. Cultures left in darkness for 4 or 6 weeks prior to an exposure to filtered blue fluorescent light for 11 days or 4 weeks produced larger and better-developed basidiocarps than did cultures in filtered red fluorescent or unfiltered white fluorescent light (78 f.c.). An 11-day exposure to light was as efficient as a 4-week exposure; 4 weeks of darkness prior to and subsequent to treatments with light were sufficient for the response. Ten minutes, 60 minutes, 24 hours, 7 days, or 14 days of high-intensity blue, red, or unfiltered white fluorescent light given after 3 weeks of darkness did not induce basidiocarp formation, but 14 days of blue light after 6 weeks of darkness effectively induced their formation. *L. trabea* apparently requires 4 to 6 weeks of growth to reach the stage of greatest sensitivity to light for basidiocarp formation. An exposure to high-intensity blue light for at least 11 to 14 days is required for this induction.

An isolate of *Trichoderma* sp. was obtained from Raymond Lukens, Department of Botany, University of Maryland. Its growth and coloration were the same in light and in darkness. Cultures sporulated abundantly in light but did not produce spores during 96 hours in the dark. The growth rate of the mycelium increased with increasing temperatures above 4.5° C. until at 32° C. the mycelium became atypical. Growth at 21° C. proved satisfactory and that temperature was used for the experiments with light. Alternation of temperature induced sporulation of certain species of fungi, but sporulation was not induced in cultures of *Trichoderma* grown in darkness at 21° C. by exposing them to 2° C. for one-half hour to 6 hours at the time of their greatest responsiveness to light. A pretreatment at 2° or 27° C. of one-half hour to 6 hours in the dark did not affect the number of spores produced at 21° C. during a subsequent exposure to light. Cultures started from small uniform-size pieces of agar with vegetative mycelium or single spores reached the stage or size for maximal response when the colony had a diameter of about 1.5 cm. This was attained after 26 or 40 hours, respectively, at 21° C. With a

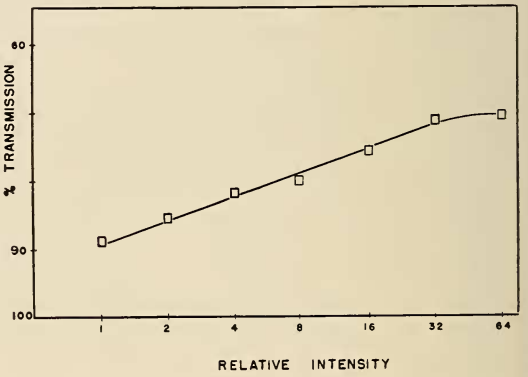

FIG. 2.—Sporulation of *Trichoderma* sp. as a function of different relative energies (value of 1 = 1.5 f.c.) of white fluorescent light given for 1 minute. Sporulation expressed in terms of percent transmission of light by spore suspension.

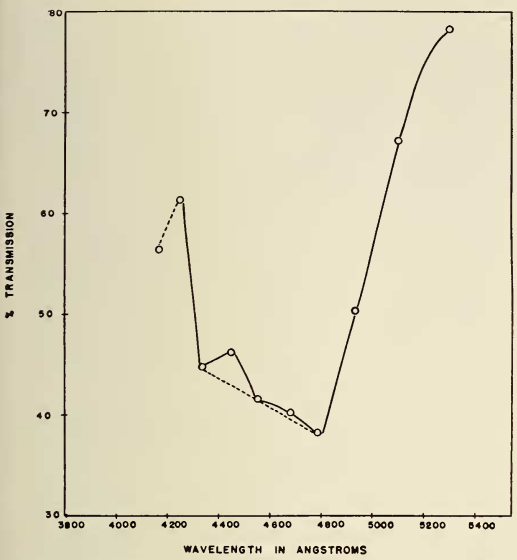

Fig. 3.—Action spectrum curve for sporulation of *Trichoderma* sp. at energies of 6×10^4 ergs/cm^2. Sporulation expressed in terms of percent transmission of light by spore suspension.

1-minute exposure to light, the number of spores increased linearly with a logarithmic increase in intensity from 1.5 to 50 f.c. of white fluorescent light (Fig. 2). At least 100 times as much energy was required for cultures of 1.0-cm diameter as for those of 1.5-cm diameter. Twenty-four hours after exposure to light the first visible sign of sporulation was the occurrence of a white ring of swollen hyphal tips 0.6 cm wide. The outer edge of the ring corresponded to the periphery of growth of the culture at the time the light was given. In an additional 24 hours this ring took on the characteristic blue-green of fully developed spores. With exposure times above saturation for sporulation, the spores matured earlier. If a portion of the ring of hyphal tips was exposed to light, sporulation was localized to those tips actually receiving light and the stimulus was not translocated to the unexposed but otherwise receptive hyphal tips (Fig. 1, lower row). Localized responses have been reported for the light-stimulated formation of pycnidia of *Physalospora obtusa* (Fulkerson, 1955) and the initiation of fruiting of *Coprinus lagopus* (Madelin, 1956).

Other workers reported that blue light was more effective than red for spore production in cultures of *Trichoderma lignorum* (Lilly and Barnett, 1951) and one isolate of *Trichoderma* sp. (Krietlow, 1938). Preliminary experiments with cellophane filters demonstrated the ineffectiveness of the red and far-red wavelengths, and this ineffectiveness was verified with the spectrograph. The action spectrum (with incident energies of 6×10^4 ergs/cm^2) showed that the most effective wavelengths for inducing sporulation were 4,300 to 4,900 Å. A sharp break occurred near 4,800 Å and sporulation was not induced by wavelengths longer than 5,200 Å (Fig. 3).

DISCUSSION

Numerous reports in the literature clearly show that fungi, although heterotrophic, require light for control of many features of their growth and reproduction. The widespread sensitivity of fungi to light is emphasized by the results of this study, in which 11 of 12 species exhibited one or more responses to light. These organisms, moreover, were selected at random as far as prior knowledge of their sensitivity to light was concerned and they were not subjected to a wide range of conditions during their culture.

The stage of development, age, or size of the fungus colony required for maximal response to light, as well as the total energy needed for a response, appears to vary with type of response and species. The amount of energy required for asexual sporulation in *Trichoderma*, for example, differed strikingly from that required for the same response in *Stemphylium*, *Botrytis* and *Penicillium*. *Trichoderma* sporulated after a short exposure to low-intensity light, and the reaction was easily saturated. These results resemble those reported for fruiting of *Coprinus lagopus* (Madelin, 1956). On the other hand, spore formation by *Stemphylium* sp. increased with increase in duration of exposure to relatively high-intensity light and saturation was difficult to attain. Blue light was the most effective for sporulation of both *Trichoderma* and *Stemphylium*—indicating that a similar pigment or pigment system absorbs the energy. The difference in the total energy requirement might be the result of differences in con-

centration of the absorbing pigment, different pathways of metabolism, or still other factors.

Sporulation of the above-mentioned fungi did not require a dark period prior to exposure to light. Responses of certain of the others appeared to be preconditioned by a dark period. For example, *Botrytis gladiolorum* responded differently depending on its age or stage of development when exposed to light. Sclerotia were not formed in cultures exposed continuously to light, but continuous light ultimately induced the greatest number of spores. Sclerotia were formed in the greatest number if, starting from a single spore, cultures received five or six days of darkness prior to a 30-minute exposure to light. It is not known whether the formation of sclerotia was preconditioned by the dark period or the dark period served to suppress the other responses and this suppression permitted the formation of sclerotia.

Ridging of *Botrytis* cultures in response to daily cycles of light and darkness occurred only when more than one cycle was used. Reports on this kind of response are rare for fungi (Sagromsky, 1952b). Such a response resembles photoperiodism in higher plants. Photoperiodic responses of higher plants, however, are controlled by red and far-red wavelength regions of the spectrum, whereas cyclic phenomena of fungi appear to be elicited by blue light.

Except for sclerotium formation in *Botrytis*, which was stimulated by red light, all responses of these fungi were promoted best by the blue region of the spectrum. The action spectrum for sporulation of *Trichoderma* showed a peak in the blue wavelengths and a sharp cutoff near 5,000 Å. These features are similar to those of other action spectra reported for fungi, such as conidiophore elongation in several isolates of *Penicillium* and *Verticillium* (Sagromsky, 1952a; Sagromsky, 1952b), sporophore elongation (Bünning, 1953) and trophocyst formation (Page, 1956) in *Pilobolus kleinii*, giant conidiophore formation in *Aspergillus giganteus* (Gardner, 1955), formation of macroconidia in *Sclerotinia fructigena* (Sagromsky, 1952b), fruiting of *Coprinus lagopus* (Borris, 1934; Madelin, 1956), and carotenoid production in *Neurospora crassa* (Zalokar, 1955). The action spectra resemble the action spectrum of phototropism and the absorption spectra of carotenoids and riboflavins. Several pigments from both of these groups, along with other pigments, have been reported in various species of fungi. Recent works (Bünning, Dorn, Schneiderhöhn, and Thorning, 1953; Zalokar, 1955), especially those on metabolic inhibitors (Page, 1956) and mutants (Cantino and Horenstein, 1956), appear to favor a flavoprotein as the absorbing pigment for such reactions to light.

Further research is required in this field to establish similarities and dissimilarities between fungi and other groups of plants, but fungi appear to have pigment systems absorbing mainly in the blue wavelengths, whereas green plants appear to possess these, for nonphotosynthetic reactions, as well as pigment systems absorbing in the red and far-red wavelengths.

SUMMARY

Light elicited the following responses in certain species of fungi: Spore production, mycelial growth and coloration, and coloration of the medium in cultures of a yellow mycelial strain of *Stemphylium* sp.; spore production, ridging and formation of sclerotia by *Botrytis gladiolorum*; and spore production by *Curvularia trifolii*, *Penicillium gladioli*, and a dark mycelial strain of *Stemphylium* sp.; formation of pycnidia by *Diplodia* sp.; basidiocarp formation by *Lenzites trabea*; formation of sporangia by *Rhizopus* sp.; formation of sclerotia by *Stromatinia gladioli*; and, the formation of sclerotiumlike bodies by *Rhizoctonia carotae*.

Spores were not formed by *Trichoderma* sp. within a 96-hour period of growth in darkness, but they were formed after a 1-minute exposure to white fluorescent light (1.5 f.c.). Alternation of temperatures neither induced sporulation nor increased the number of spores produced upon subsequent exposure to light. Spores were localized to that portion of the ring of hyphal tips exposed to light. Spore production increased linearly with a logarithmic increase in intensity from 1.5 to 50 f.c. of white fluorescent

light. The action spectrum for sporulation showed a peak of response to wavelengths of 4,300 to 4,900 Å, a sharp break near 4,800 Å, and ineffectiveness of wavelengths longer than 5,200 Å.

With a possible exception of red-light-induced sclerotium formation in *Botrytis gladiolorum*, these responses were induced with the shorter (less than 5,200 Å) wavelengths of the visible spectrum.

These findings corroborate the researches of other workers concerning the greater effectiveness of the shorter wavelengths of the visible spectrum and the widespread importance of light in the life cycle of fungi.

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